VIEW POINT

Essential *In vivo* **Safety /Tox studies** to move your molecule from Target to IND successfully

Syngene

Putting Science to Work

Introduction

According to research, 85% of new drugs fail during clinical trials (Phase-1 to FDA approval). The reasons for failure include poor solubility, life-threatening or other undesirable side effects, poor biodistribution by the proposed clinical route of administration, poor efficacy in early clinical trials, and so on.

For a molecule to progress smoothly from early-stage discovery to the investigational new drug (IND) phase and further to successful clinical trials, the data from preclinical trials must be accurate, reliable, and based on the best suitable and comparable model available to the target population. This means the IND or drug product must undergo a series of robust tests and experiments as per the focused indication and regulatory guidelines (FDA/EMA etc.).

In this point of view, we discuss how Syngene's expertise in *In vivo* Safety /Tox studies can help move your molecule from early discovery to IND stage successfully. With 27 years of scientific experience, working across 400+ global clients, we have been able to create a roadmap for IND-enabling preclinical studies, supported at each stage by a strong supply chain, logistics, program management, and data security. Read on.

Syngene's capability in preclinical Safety/Tox studies

Syngene's integrated drug development and assessment capabilities put it at the forefront of discovering new therapies for various diseases. We can support you from the very start of your drug development journey to the clinic and further to commercialization. We have extensive experience working in this space, including in the field of IND-enabling safety and toxicity studies. So far, we have supported 400+ global clients from early discovery to development across multiple targets.

Our expertise in Safety/Tox studies allows us to guide you through the various requirements leading up to IND submission (Figure 1). These include:

- Assessment of pharmacokinetics such as dose formulation in rodent and non-rodent species, the bioavailability of the molecule, and assessment covering gender differences
- Maximum tolerated dose (MTD) studies and dose range-finding (DRF) studies across rodent and non-rodent species
- Assessment of genetic toxicity using bacterial reverse mutation test, chromosomal aberration test, and micronucleus test. Also, GLP toxicology studies in rats and non-rodents.

PK	MTD	DRF	GLP Toxicology
Pharmacokinetics • Dose Formulation • Rodent and nonrodent species • AUC • T_{max} • C_{max} • Bioavailability • Gender difference	**Single/multiple dose** **MTD study** • Rodent and nonrodent species • Dose escalation/3-4 ascending doses • Rat: 3/sex/group **Non-rodent: 1Male+1Female** • DRF studies: 7/14 days with TK • Rat study: 5/sex/group • Non-rodent: 2/sex/group • 1 control+ 2 or 3 dose levels		**Genetic toxicology** • Bacterial Reverse Mutation Test • Chromosomal Aberration Test • Micronucleus Test **GLP toxicology study** • Rat study: 10/sex/group • Non-rodent: 3/sex/group • 1 control+ 3 dose levels

Figure 1: Flow of preclinical studies

We also have a range of species for testing target molecules. While a majority of the studies recommend using rats and dogs, other species can also be used if data suggests that they are more biologically relevant. For clients that prefer the use of non-rodent species, we offer preclinical studies which can be conducted in non-human primates such as minipigs.

Pivotal GLP tox study

A typical study design for pivotal GLP studies for rodents and non-rodents involves a dosing phase of 28 days, followed by a recovery phase of 14 days or 28 days. The rodents/non-rodents are divided into four groups (control, low dose, mid-dose, and high dose), each consisting of an equal number of males and females with standard observations/ endpoints. Toxicokinetic is integrated into the pivotal GLP tox study. The parameters observed include morbidity and mortality (daily checks); pre and post-dose clinical signs (pre and post-dose); ophthalmic examination; bodyweight; food consumption; clinical pathology (main groups and recovery groups); necropsy and gross examination; histology of all organs including injection site(s), if any; dose formulation concentration analysis and bioanalysis and toxicokinetics.

Safety Pharmacological studies

We conduct Pharmacological studies using the Irwin test, Respiratory Function test, Cardiovascular Safety Pharmacology test, and *in vitro* hERG assays.

- Irwin test: We conduct this study to test the pharmacological effects of target molecules on the central nervous system in rodents. We use it to assess the functionality and toxicity of chlorpromazine, triadimefon, trimethyltin, and acrylamide. The test is also used to assess many bodily functions, such as respiratory pattern, mobility, gait, salivation, pupil reflex, and motor activity (Figure 2)
- Respiratory function test: A special plethysmograph chamber is used to assess the respiratory properties of rodents. The parameters monitored include Ti-inspiration time, Te-expiratory time, PiF-peak inspiratory flow, PeF-Peak expiratory flow, TV-Tidal volume, MV-Minute volume, F-Respiratory rate, RT-Relaxation time
- Cardiovascular safety pharmacology: Telemetry [Jacketed/Invasive] using non-rodents
- *In vitro* human Ether-a-go-go Related Gene (hERG) assay: Patch clamp

Biologics Studies

To test a target molecule in an animal model, we pay special attention to the following:

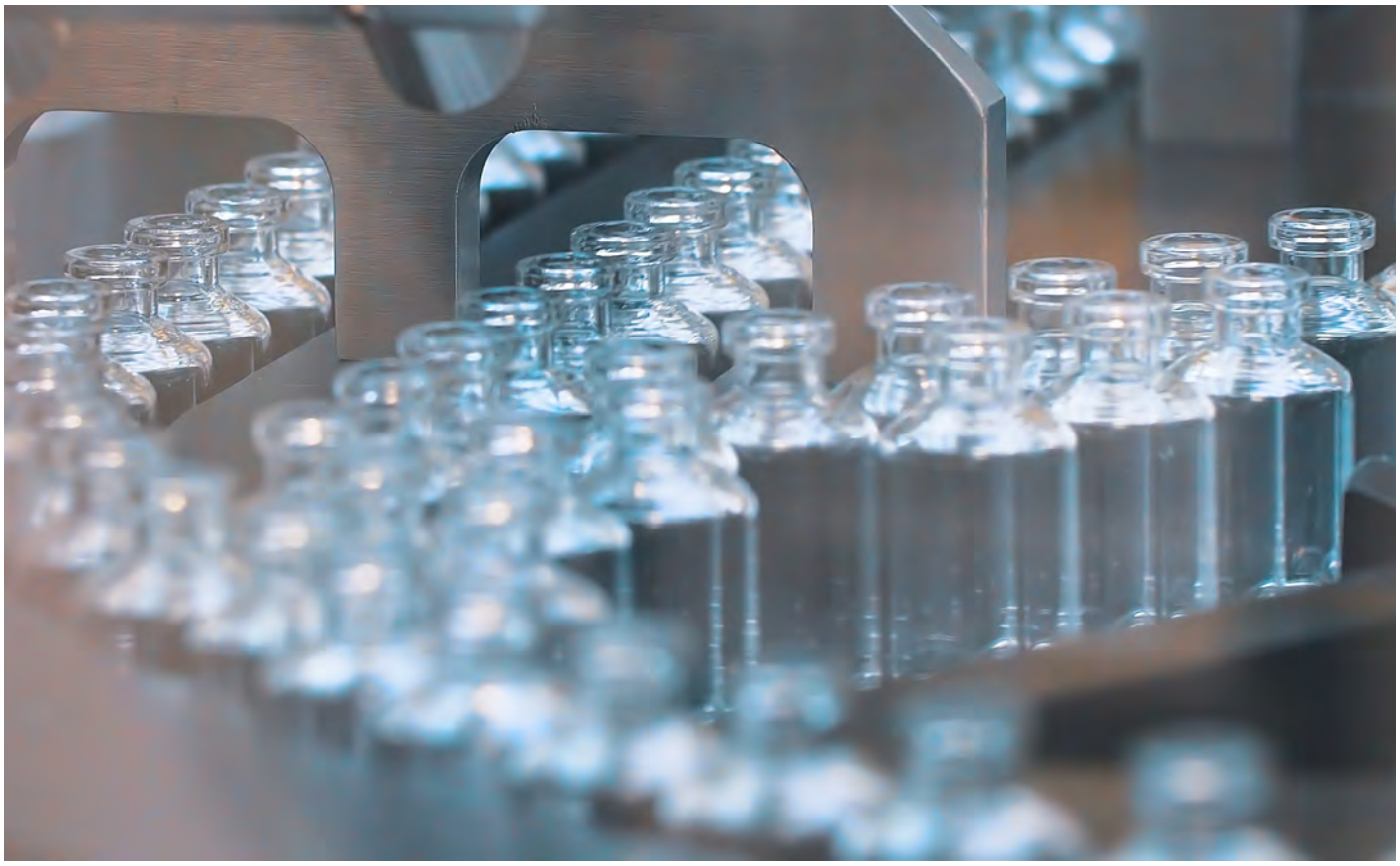
1. Selection of relevant animal species
    - Studies are done in relevant species, i.e., species that demonstrate pharmacological effects
    - With biologics, non-human primates (NHPs) are often the only relevant species available for testing
    - If a second species is relevant, we evaluate them as well
    - No relevant animal model other than transgenic strain expressing human receptor is considered for studies
2. Dose, route of administration, and treatment regimen
    - High dose, 10X over maximum exposure, tested to establish an adequate safety margin.
    - Route/frequency of administration kept the same as that proposed for clinical use
    - Conducting pivotal 28-day repeated dose toxicology study in relevant animal species

Vaccine Studies

Our vaccine study design comprises a 43-day dosing phase, followed by a 28-day recovery period. Animals are assessed for clinical pathology, organ weights, gross pathology, histology of organs, immunogenicity, local tolerance, and other clinical signs.

We also conduct vaccine prenatal developmental toxicity study design covering the following:

- Clinical signs, local tolerance, body weight, and food consumption
- Cesarean section: Gestation day 20
- Maternal data: Uterine weight, # corpora lutea, implantation sites, early and late resorptions, pre-and post-implantation losses
- Litter data: Total no. of fetuses (live and dead), litter weight, Sex ratio (M: F)
- Fetal evaluations: External, visceral, and skeletal malformations. Classified as normal variants, minor anomalies, and major malformations
- Immunogenicity: Prior to dosing and at termination



IND GLP Tox and Safety Pharmacology studies

We offer the following IND GLP Tox and Safety Pharmacology studies across a 26-week timeline (Figure 2):

	Timelines (weeks)																								
GLP studies	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
Analytical method development and validation for *In vivo* studies for dose confirmation analysis																										
Bioanalytical method validation – rat plasma																										
Bio-analytical method validation – nonrodent plasma																										
28-day repeat dose tox study with TK and 14-day recovery period in rats																										
In vivo micronucleus test in rat																										
Analytical validation – *In vitro* studies																										
In vitro Ames test																										
In vitro chromosomal aberration test																										
Analytical transfer validation for dose confirmation analysis-rats/ non-rodent																										
28-day repeat dose tox study with TK and 14-day recovery period in non-rodent																										
CNS-safety pharmacology – rat																										
Respiratory safety pharmacology – rat																										
Cardiovascular telemetry study – nonrodent																										
In vitro hERG Assay																										

Figure 2: IND GLP tox and safety pharmacology—indicative timelines

GLP: Good Laboratory Practices; A – N studies as mentioned before this figure.

Conclusion

Syngene has significant expertise in preclinical studies across species (rodent/non-rodent) and a deep understanding of the molecular assessment process. Our studies are performed in compliance with good laboratory practice and good scientific practices (GLP/GSP) to ensure reliability and reproducibility of results that satisfy stringent regulatory guidelines (i.e., FDA/EMA).

By partnering with us, biopharma companies can greatly increase their chances of moving their molecules from Target to IND successfully. Further, Syngene has also significantly optimized the various steps involved in taking a molecule from lab to human trials and commercialization. With this, biopharma can reap the benefits of accelerated timelines for synthesizing new drugs.

About the Author

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Mohan Krishnappa is a Lead toxicologist with 10+ years of industry experience in the field of GLP preclinical toxicology research and toxicology risk assessment of health care products and industrial chemicals. He is proficient in the execution of GLP preclinical toxicology/safety studies in accordance with global regulations/ compliance for submissions to regulatory agencies, namely EMEA, USFDA, and MHRA. He has served as study director/toxicologist/scientific director/study monitor for several toxicology projects involving new chemical entities, generics, pivotal GLP toxicology studies, PK/TK, juvenile and DART toxicity studies, impurity qualification studies, biocompatibility testing of medical devices, including IND enabling toxicology and safety pharmacology programs. Currently, he is associated with Safety Assessment operating unit at Syngene as Associate Research Director.

To know more about our Safety Assessment services or to contact our experts, please click here

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About Syngene

Syngene International Ltd. (BSE: 539268, NSE: SYNGENE, ISIN: INE398R01022) is an integrated research, development and manufacturing services company serving the global pharmaceutical, biotechnology, nutrition, animal health, consumer goods and specialty chemical sectors. Syngene's more than 4700 scientists offer both skills and the capacity to deliver great science, robust data management and IP security and quality manufacturing at speed to improve time-to-market and lower the cost of innovation. With a combination of dedicated research facilities for Amgen, Baxter and Bristol-Myers Squibb as well as 2 Mn sq. ft of specialist discovery, development and manufacturing facilities, Syngene works with biotech companies pursuing leading-edge science as well as multinationals, including GSK and Merck KGaA.

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